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Immunotherapy of *Cryptococcus* infections

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Abstract

Despite appropriate antifungal treatment, the management of cryptococcal disease remains challenging, especially in immunocompromised patients, such as human immunodeficiency virus-infected individuals and solid organ transplant recipients. During the past two decades, our knowledge of host immune responses against *Cryptococcus* spp. has been greatly advanced, and the role of immunomodulation in augmenting the response to infection has been investigated. In particular, the role of 'protective' Th1 (tumour necrosis factor-α, interferon (IFN)-γ, interleukin (IL)-12, and IL-18) and Th17 (IL-23 and IL-17) and 'non-protective' Th2 (IL-4, IL-10, and IL-13) cytokines has been extensively studied *in vitro* and in animal models of cryptococcal infection. Immunomodulation with monoclonal antibodies against the capsular polysaccharide glucuronoxylomannan, glucosylceramides, melanin and β-glucan and, lately, with radioimmunotherapy has also yielded promising results in animal models. As a balance between sufficiently protective Th1 responses and excessive inflammation is important for optimal outcome, the effect of immunotherapy may range from beneficial to deleterious, depending on factors related to the host, the infecting organism, and the immunomodulatory regimen. Clinical evidence supporting immunomodulation in patients with cryptococcal infection remains too limited to allow firm recommendations. Limited human data suggest a role for IFN-γ. Identification of surrogate markers characterizing patients' immunological status could possibly suggest candidate patients for immunotherapy and the type of immunomodulation to be administered.

Keywords: *Cryptococcus*, cytokines, HIV, immunotherapy, interferon-γ, monoclonal antibodies
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Introduction

The genus *Cryptococcus* includes 37 recognized basidiomycetous yeast species [1]. Human disease is mainly caused by *Cryptococcus neoformans* and *Cryptococcus gattii* [1]; other species, such as *Cryptococcus laurentii* and *Cryptococcus albidus*, have rarely been reported to affect humans [2]. *C. neoformans* has a global geographical distribution; *C. gattii* infections have been reported from tropical and subtropical regions and from the outbreak that emerged in the temperate area of British Columbia in 1999 and then expanded towards Washington, Oregon, and California [1–4]. *C. neoformans* affects mainly immunocompromised individuals, such as those with advanced human immunodeficiency virus (HIV) infection, solid organ transplant recipients, and patients receiving immunosuppressive agents, including high-dose steroids and monoclonal antibodies (mAbs) [1]. Indeed, cryptococcal meningitis is the leading cause of death in HIV-infected individuals worldwide, with a global burden of approximately 1 million cases occurring each year [5]. It appears that most infections in HIV patients are caused by *C. neoformans* var. *grubii* (serotype A), and fewer are caused by *C. neoformans* var. *neoformans* (serotype D) [6,7]. *C. gattii*, in contrast, has been associated with infections in immunocompetent individuals [3].

The management of cryptococcal disease remains challenging, especially in immunocompromised patients. The combination of highly active antiretroviral therapy with antifungal treatment in HIV-infected individuals has reduced the 12-month and 24-month mortality rates to 25% and 34%, respectively, as compared with corresponding rates of 54%

and 76% in the period before highly active antiretroviral therapy. However, the 3-month mortality rate of acute cryptococcal meningoencephalitis in HIV-infected patients still approximates 20% [8]. Similarly, the 3-month mortality rate of solid organ transplant recipients with cryptococcosis in a recent retrospective study was 28.6% [9]. These data highlight the need for optimization of treatment of *Cryptococcus* infections by means of newer approaches and potential adjunctive therapies. In this context, the potential for restoration and optimal enhancement of host defence mechanisms through immunomodulation has attracted wide scientific interest. The present review will concisely present human immune responses against *Cryptococcus* and then discuss recent developments in the immunotherapy of cryptococcal infections.

Overview of Immune Responses Against *Cryptococcus*

Cryptococcus enters the human body by inhalation. Following adherence to and internalization of the organisms by pulmonary epithelial cells, pulmonary alveolar macrophages, and dendritic cells, infection is usually contained, and leads to mild or no symptoms in the majority of immunocompetent individuals. If the yeast evades the host immune mechanisms, as is the case in immunocompromised patients, it disseminates to other organs, particularly the brain [1]. *Cryptococcus* possesses a number of mechanisms by which it overcomes host responses; these include the formation of a polysaccharide capsule, the production of melanin and virulence-related enzymes (such as phospholipase B1 and urease), and its ability to grow at 37°C and live as an intracellular pathogen in host cells [10,11].

The innate immune response against *Cryptococcus* encompasses both the complement system and phagocytic effector cells (dendritic cells, macrophages, and neutrophils). The role of the complement system is to stimulate chemotaxis of phagocytic cells and opsonize the pathogen in order to facilitate phagocytosis [11]. *In vitro* data suggest a key role for the alternative complement pathway in cryptococcosis [12,13]; however, optimal opsonization requires the contribution of the classical pathway as well [14]. Both complement activation and phagocytosis appear to be inhibited by the cryptococcal polysaccharide capsule [10].

Phagocytosis of cryptococcal cells follows direct recognition of conserved yeast structures (such as the capsular polysaccharide glucuronoxylomannan and mannoproteins) [15,16] or opsonization of yeast cells via complement or antibodies [17]. Dendritic cells are able to phagocytose *C. neoformans*, and subsequently act as major antigen-presenting cells and modulators of adaptive immune responses [18,19]. It appears that dendritic cells are more potent inducers of T-cell responses than macrophages [18,20]. The latter play a complicated but not always beneficial role in the host response during cryptococcal infection. Indeed, following phagocytosis by the macrophages, *C. neoformans* is able to survive and replicate in the phagolysosomes, thus escaping other protective host immune responses and maintaining a state of latent infection [21–24]. Subsequently, *Cryptococcus* can exit macrophages and remain in the extracellular environment (causing, for example, fungaemia) or be transferred from one macrophage to another, maintaining the latent phase [25,26]. Neutrophils exert their antifungal activity both by direct oxidative killing of cryptococcal cells and by production of antimicrobial peptides, such as the defensins [27–31].

The adaptive immune response against *Cryptococcus* includes antibody-mediated and cell-mediated responses. During cryptococcal infection, both protective and non-protective anticryptococcal antibodies may be formed [32–34]. Protective antibodies activate the classical complement pathway or opsonize yeast cells through complement-independent mechanisms [35,36]. Part of their protective activity also appears to be exerted through interaction with elements of cell-mediated immunity [11]. Cell-mediated immune responses against *Cryptococcus* include both direct cytotoxic effects and regulation of cytokine production. Direct cytotoxic effects are exerted by natural killer (NK), $CD4^+$ and $CD8^+$ cells, and are mediated by secreted proteins (perforin and granulysin) [11,37–40]. Cytokine production involves protective Th1 (tumour necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-12, and IL-18) and Th17 (IL-23 and IL-17) responses and non-protective Th2 (IL-4 and IL-13) responses. A Th1–Th17 cytokine profile is associated with increased phagocytic activity and inhibition of *Cryptococcus* proliferation [41–44], whereas a Th2 profile is associated with decreased ability of the host to effectively control the infection [45,46]. However, an exaggerated Th1 response may lead to extensive inflammation and suboptimal outcome for the host; sustainment of an optimal Th1–Th2 balance is now known to be mediated by $\gamma\delta$ T-cells [47].

Immunotherapy of Cryptococcal Infections: Preclinical Studies

In vitro and *in vivo* studies on the immunomodulation of cryptococcal infections have mainly focused on two fields: the role of cytokines, and the role of mAbs (Table 1).

TABLE 1. Immunotherapy of cryptococcal infections: comparative data of preclinical and clinical studies

Immunomodulatory agent	Preclinical studies	Clinical studies
Th1 cytokines	Protective effect of IL-12 and IL-18 in animal models [48–53] Protective effect of IFN-γ in animal models; synergism with antifungal drugs [54–58] Inhibitory effect of IFN-γ in fungistatic activity of human macrophages against *Cryptococcus* [59,60] Stimulatory/protective effect of TNF-α and IL-2 [61–64]	Correlation between TNF-α, IFN-γ and IL-6 levels and survival/clearance of infection in HIV-infected patients with cryptococcal meningitis [93] Increased levels of TNF-α, IL-6 and IL-8 in the CSF of patients with meningeal vs. extrameningeal infection and in HIV-negative vs. HIV-positive patients [95] Phase 2 study of rIFN-γ1b as adjunctive therapy in HIV-infected patients with cryptococcal meningitis: trend towards more rapid sterilization of CSF and improved combined mycological–clinical response [96]
Monoclonal antibodies (mAbs)	Protective effect of mAbs targeting glucuronoxylomannan, glucosylceramides, melanin or β-glucan *in vitro* and in animal models [65–87] Protective effect of radiolabelled mAbs (^{188}Re-labelled 18B7 and ^{213}Bi-labelled 18B7) in animal models [88–92]	Phase 1 dose escalation study of mAb 18B7 administration in HIV-infected patients with cryptococcal meningitis: modest and transient reduction of serum cryptococcal antigen titres; doses up to 1 mg/kg well tolerated [98]

CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; rIFN, recombinant interferon; TNF, tumour necrosis factor.

Preclinical studies involving cytokines

A number of studies have demonstrated the protective effect of Th1 cytokines during experimental cryptococcal infection.

In a murine model of *C. neoformans* pulmonary and disseminated infection, early administration of IL-12 resulted in marked infiltration of inflammatory cells and a reduction in the number of viable yeast cells in the lung, suppressed dissemination to the brain, and increased survival rate of infected mice. IL-12 treatment was associated with induction of IFN-γ production in the lungs of infected mice. Late (7 days after inoculation) administration of IL-12 was not associated with a protective effect in this model [48]. In another murine model of systemic cryptococcosis, administration of IL-12 significantly reduced the level of brain and liver infection (quantified as CFUs of *C. neoformans*); in the same study, IL-12 enhanced the antifungal effect of fluconazole [49]. In a murine model of chronic *C. neoformans* infection, long-term administration of exogenous IL-12 reduced the fungal burden in various organs and prevented the death of infected mice [50].

The protective effect of another Th1 cytokine, IL-18, has also been demonstrated in a murine model of pulmonary and disseminated cryptococcosis. Treatment with recombinant murine IL-18 in this study enhanced elimination of *Cryptococcus* from the lungs, inhibited dissemination to the central nervous system, and prolonged the survival of infected mice. IL-18 treatment was associated with increased levels of serum IFN-γ; administration of neutralizing anti-IFN-γ antibodies abrogated the protective effect of IL-18 [51]. A synergistic interaction of IL-18 with IL-12 has been shown in the induction of anticryptococcal activity of NK cells [52]. This synergistic interaction was subsequently demonstrated in a murine model of pulmonary and disseminated cryptococcosis, where combined IL-18 and IL-12 treatment prolonged the survival of infected mice and reduced fungal load in the lung and brain; this effect was mediated by enhancement of IFN-γ production by NK and $\gamma\delta$ T-cells and suppression of IL-4 production in the lungs [53].

The contribution of IFN-γ in controlling cryptococcal infection has been shown in murine models of lung and disseminated cryptococcosis, where IFN-γ administration prolonged survival and reduced fungal burden in the lungs and brain [54–58]. In some of these studies, IFN-γ also enhanced the efficacy of amphotericin B treatment of mice in terms of survival prolongation and reduction of CFUs in various organs (brain, liver, kidneys, and lungs) [54,56,57]. The protective effect of IFN-γ in mice against *Cryptococcus* appears, however, to contradict the results of *in vitro* studies involving human cells. Addition of IFN-γ resulted in loss of growth inhibition of *C. neoformans* by human peripheral blood cultured monocyte-derived macrophages [59]. Similarly, IFN-γ decreased the fungistatic capacity of human alveolar macrophages against *C. neoformans in vitro* [60]. These studies suggest that the regulation of the anticryptococcal functions of macrophages by cytokines may differ between humans and mice.

The role of other cytokines in enhancing immune responses against *Cryptococcus* has been demonstrated in a number of *in vitro* and *in vivo* studies. Addition of TNF-α increased the nitric oxide-mediated anticryptococcal activity of murine macrophages *in vitro* [61]. The combination of TNF-α with granulocyte–macrophage colony-stimulating factor acted synergistically in enhancing the complement-dependent phagocytosis of *C. neoformans* by murine macrophages [62]. In a murine model of disseminated cryptococcosis, the combination of an agonist antibody against CD40 (anti-CD40), a member of the TNF-α receptor family, with IL-2 prolonged the survival of infected mice and decreased yeast burdens in brain and kidney; this beneficial effect was mediated through increased IFN-γ production [63]. The same

combination (anti-CD40 and IL-2) also prolonged the survival of mice infected intracerebrally with *C. neoformans*; this effect correlated with activation of microglial cells. IFN-γ was again an important mediator of the anticryptococcal activity of this combination [64].

Preclinical studies involving mAbs

The immunomodulatory effect of mAbs in the course of experimental cryptococcal infection has been demonstrated and extensively studied over the last three decades. Most of these studies have utilized mAbs against glucuronoxylomannan [65–67]; mAbs targeting glucosylceramides [68,69], melanin [70] and β-glucan [71] have also been studied. The immunomodulatory effects of mAbs appear to be exerted in a complex and pleiotropic manner through enhancement of opsonization and phagocytosis [71,72], direct inhibition of fungal cell growth [68,70,71], reduction of capsule thickness [71], and, importantly, modulation of cytokine production and inflammation [73–75].

Passive immunization with mAbs in the case of cryptococcal infection may result in protection, non-protection, or even disease enhancement, depending on the mAb dose and other characteristics, as well as host immune parameters [67,76]. In particular, the outcome may be affected by the relationship between the amount of mAb administered and the fungal inoculum; under certain conditions, a prozone-like effect has been observed, with high mAb doses being less effective than smaller mAb doses [72,77]. Among other factors, the antibody subclass [78] and isotype [79,80], epitope specificity [34], availability of T-cells [81] and B-cells [75] in the host, presence of nitric oxide [82] and production of Th1 and Th2 cytokines [73] have been shown to influence the outcome of passive immunization studies.

The protective effect of mAbs in murine models of cryptococcal infection has mostly consisted of prolongation of survival in treated vs. untreated animals [67,69,70,75,79,82–85]. Administration of mAbs also has been associated with a reduction of fungal burden in the lungs [70,86], brain [70,71,84], liver [71,87] and spleen [86,87] of treated mice. Prolonged survival in animals treated with mAbs was more frequently associated with downregulation [69,74,75] than with enhancement [82] of the inflammatory response.

Over the past several years, a novel approach, radioimmunotherapy, has been developed as an adjunctive immunomodulatory treatment for cryptococcal infections. The concept of radioimmunotherapy is to use radiolabelled mAbs in order to deliver cytotoxic radiation to fungal cells; in this way, an antibody without inherent anticryptococcal activity can be converted into a fungicidal molecule. Indeed, in a murine model of systemic cryptococcosis, administration of β-emitter (^{188}Re)-labelled or α-emitter (^{213}Bi)-labelled mAb 18B7 was associated with prolonged survival and reduced fungal burden in the lungs and brain of treated animals as compared with controls, without apparent toxicity [88]. The protective effect of ^{188}Re-labelled 18B7 and ^{213}Bi-labelled 18B7 in cryptococcal infection appears to be mediated through promotion of apoptosis-like death of yeast cells, a decrease in the size of the cryptococcal capsule, a synergistic interaction with macrophages, and modulation of the inflammatory response [89]. Radioimmunotherapy was shown to be relatively safe in mice, and maximum tolerated doses were determined. With these doses, only transient haematological toxicity and no evidence of pulmonary fibrosis were observed [90]. In more recent studies, radioimmunotherapy with ^{188}Re-labelled 18B7 (which has a much longer half-life than ^{213}Bi-labelled 18B7) increased survival in mice infected with a high inoculum of *C. neoformans* and did not select for yeast cells resistant to radiation [91]. Furthermore, radioimmunotherapy with ^{213}Bi-labelled 18B7 was more effective than amphotericin B in decreasing fungal burden in the lungs and brain of mice infected with *C. neoformans* [92].

Immunotherapy of Cryptococcal Infections: Clinical Studies

In contrast to the abundance of *in vitro* and *in vivo* studies on the immunomodulation of cryptococcal infections, relevant clinical data are very limited (Table 1).

In a series of 62 patients receiving antifungal therapy for HIV-associated cryptococcal meningitis, the concentrations of TNF-α, IFN-γ, IL-6 and IL-8 in the cerebrospinal fluid (CSF) were significantly higher in survivors than in non-survivors. Negative correlations were found between log TNF-α, IFN-γ and IL-6 levels and the baseline CFU level of *Cryptococcus* in the CSF. Log TNF-α, IFN-γ, IL-6 and granulocyte colony-stimulating factor concentrations in the CSF correlated positively with the rate of fall of log CFU. In multivariate analysis, the two factors independently associated with the rate of clearance of infection were treatment group and log IFN-γ level [93]. The same group of investigators reported very low concentrations of IFN-γ, TNF-α and IL-6 in the CSF of an apparently immunocompetent, HIV-seronegative patient with *C. gattii* meningitis [94], as compared with the levels found in patients with HIV-related *C. neoformans* meningitis of the previous study [93]. In the same patient, the CSF concentration of IL-10 was very high; taken together, these data suggest a distorted immune response to *C. gattii* exposure, allowing progression to meningitis [94].

In another study, the levels of TNF-α, IL-6, IL-8, IL-10 and soluble TNF receptor II were measured in the CSF of 51 HIV-positive and seven HIV-negative patients with culture-confirmed cryptococcosis. With the exception of IL-10, the concentrations of the above mediators were higher in patients with meningeal cryptococcosis than in those with extrameningeal infection or controls. Among meningitis patients, levels of all mediators, except soluble TNF receptor II, were higher in HIV-negative patients than in those who were HIV-positive [95].

In a phase 2, double-blind, placebo-controlled study of adjuvant recombinant IFN (rIFN)-γ1b in 75 HIV-infected patients with acute cryptococcal meningitis, 2-week CSF culture conversion (from positive to negative) occurred in 13% of placebo recipients vs. 36% of patients receiving 100 μg of rIFN-γ1b three times weekly (p 0.072) and 32% of patients receiving 200 μg of rIFN-γ1b three times weekly (p 0.139). These results suggest a trend towards more rapid sterilization of CSF in rIFN-γ1b recipients. Combined mycological–clinical success (negative CSF culture and clinical stability or improvement at 2 weeks) was observed in 26% of rIFN-γ1b recipients as compared with 8% of controls (p 0.078). Treatment with rIFN-γ1b was well tolerated [96].

Ex vivo studies in two patients with severe cryptococcal meningitis and idiopathic $CD4^+$ lymphopenia revealed reduced production of IFN-γ and TNF-α but not IL-10 in comparison with healthy controls. In one of these patients with clinical deterioration despite appropriate antifungal treatment, adjunctive immunotherapy with rIFN-γ was commenced (50 $\mu g/m^2$ three times weekly). Four weeks later, clinical recovery was almost complete and CSF culture was negative; because of persistence of low titres of cryptococcal antigen, mild CSF leukocytosis, and hypoglycorrhachia, however, treatment with IFN-γ was continued for 2 years and subsequently reduced to 50 $\mu g/m^2$ once weekly [97].

In a phase 1 dose escalation study, the safety and maximum tolerated dose of mAb 18B7 were investigated in HIV-infected patients who had been successfully treated for cryptococcal meningitis. The doses studied ranged from 0.01 to 2 mg/kg of body weight as a single infusion. A modest reduction in serum cryptococcal antigen titres occurred in the cohorts receiving doses of 1 and 2 mg/kg; titres tended to return to baseline measurements by week 12 from the day of infusion. Doses up to 1 mg/kg were well tolerated [98].

Conclusions

Our knowledge of host defences against cryptococcal infections has advanced greatly over the past 40 years. Although the types of immune defect that predispose to progression from infection to disease have been elucidated, there are still questions to answer; for example, what are the host factors leading to development of disease caused by *C. gattii* in apparently healthy individuals? The effect of immunomodulatory interventions, with mainly cytokines and mAbs, has been extensively studied *in vitro* and *in vivo*. It is now understood that the outcome of immunotherapy may range from beneficial to deleterious, depending on factors related to the host, the infecting organism, and the immunomodulatory regimen employed. The importance of the balance between sufficiently protective Th1 responses and excessive inflammation has also been elucidated. Newer approaches, such as radioimmunotherapy, provide a promising avenue for therapeutic alternatives.

Despite this progress, the currently published clinical data on the adjunctive use of immunotherapy in patients with cryptococcal infections are too limited to lead to firm recommendations. In the 2010 update of the clinical practice guidelines for the management of cryptococcal disease by the Infectious Diseases Society of America, a level B-III recommendation is made that addition of rIFN-γ to the standard antifungal regimen can be considered in patients with persistent cryptococcal infection ('persistent' being defined as positive CSF cultures after 4 weeks of antifungal therapy). The recommended regimen is 100 $\mu g/m^2$ of IFN-γ for patients weighing $\geq$50 kg, and 50 $\mu g/m^2$ of IFN-γ for those weighing <50 kg, three times weekly for 10 weeks. The usefulness of rIFN-γ in patients with *C. gattii* infection remains uncertain (C-III) [99].

Patients with cryptococcal disease are likely to be immunocompromised, but may well also be in a state of immune reconstitution. In the latter case, administration of a proinflammatory immunomodulator would probably lead to an excessive inflammatory response and a poor outcome; instead, the use of an anti-inflammatory mediator would probably be beneficial [100]. In this context, the identification and validation of surrogate markers characterizing the patients' immunological status would help to determine which patients could benefit from immunotherapy and what type of immunomodulation should be administered. Such surrogate markers would probably improve the quality of relevant clinical studies, eventually leading to valid conclusions and optimal management of cryptococcal disease.

Each of the major immunotherapeutic approaches (passive immunization, active immunization, and cytokine augmentation of effector cells) holds promise for advancing the treatment and prevention of cryptococcosis. Among the promising directions for passive immunotherapy of cryptococcosis is the use of targeted radioimmunotherapy [92]. The fact that radioimmunotherapy was more effective than

antifungal chemotherapy is particularly promising for the treatment of refractory disease in profoundly compromised hosts. From a broader public health perspective, stimulation of humoral and cellular immune responses following active vaccination with antigen-loaded glucan particles [101] has the potential to be widely applied for immunization against *C. neoformans*. With the use of glycoantigens from *C. neoformans* [20] that are loaded into glucan particles, dendritic cell, cellular and humoral immune systems may be activated to provide protection against cryptococcosis. Given the overwhelming number of cases and the devastating effect of cryptococcosis worldwide, a vaccine strategy should clearly play an important role in reducing the morbidity and mortality resulting from this infection. Finally, cytokine augmentation remains a promising modality. Given the encouraging effect of rIFN-γ1b in treatment of acute cryptococcal meningitis in a relatively small phase 2 study [96], the potential benefits of this strategy warrant further investigation in a larger clinical trial. Alternatively, consideration of other cytokines, such as IL-18, an important regulator of innate and acquired immunity, with demonstrated *in vivo* effects against experimental cryptococcosis, may also open new clinical options for immunotherapy.

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References

1. Li SS, Mody CH. *Cryptococcus*. *Proc Am Thorac Soc* 2010; 7: 186–196.
2. Khawcharoenporn T, Apisarnthanarak A, Mundy LM. Non-neoformans cryptococcal infections: a systematic review. *Infection* 2007; 35: 51–58.
3. Galanis E, Macdougall L. Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999–2007. *Emerg Infect Dis* 2010; 16: 251–257.
4. Byrnes EJ III, Marr KA. The outbreak of *Cryptococcus gattii* in Western North America: epidemiology and clinical issues. *Curr Infect Dis Rep* 2011; 13: 256–261.
5. Park BJ, Wannemuehler KA, Marston BJ *et al.* Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009; 23: 525–530.
6. Day JN, Hoang TN, Duong AV *et al.* Most cases of cryptococcal meningitis in HIV-uninfected patients in Vietnam are due to a distinct amplified fragment length polymorphism-defined cluster of *Cryptococcus neoformans* var. grubii VN1. *J Clin Microbiol* 2011; 49: 658–664.
7. Miglia KJ, Govender NP, Rossouw J *et al.* Analyses of pediatric isolates of *Cryptococcus neoformans* from South Africa. *J Clin Microbiol* 2011; 49: 307–314.
8. Lortholary O, Poizat G, Zeller V *et al.* Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS* 2006; 20: 2183–2191.
9. Neofytos D, Fishman JA, Horn D *et al.* Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. *Transpl Infect Dis* 2010; 12: 220–229.
10. Zaragoza O, Rodrigues ML, De Jesus M *et al.* The capsule of the fungal pathogen *Cryptococcus neoformans*. *Adv Appl Microbiol* 2009; 68: 133–216.
11. Voelz K, May RC. Cryptococcal interactions with the host immune system. *Eukaryot Cell* 2010; 9: 835–846.
12. Diamond RD, May JE, Kane M *et al.* The role of late complement components and the alternate complement pathway in experimental cryptococcosis. *Proc Soc Exp Biol Med* 1973; 144: 312–315.
13. Kozel TR, Wilson MA, Murphy JW. Early events in initiation of alternative complement pathway activation by the capsule of *Cryptococcus neoformans*. *Infect Immun* 1991; 59: 3101–3110.
14. Diamond RD, May JE, Kane MA *et al.* The role of the classical and alternate complement pathways in host defenses against *Cryptococcus neoformans* infection. *J Immunol* 1974; 112: 2260–2270.
15. Shoham S, Huang C, Chen JM *et al.* Toll-like receptor 4 mediates intracellular signaling without TNF-alpha release in response to *Cryptococcus neoformans* polysaccharide capsule. *J Immunol* 2001; 166: 4620–4626.
16. Pietrella D, Corbucci C, Perito S *et al.* Mannoproteins from *Cryptococcus neoformans* promote dendritic cell maturation and activation. *Infect Immun* 2005; 73: 820–827.
17. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS—100 - years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 1995; 8: 515–548.
18. Syme RM, Spurrell JC, Amankwah EK *et al.* Primary dendritic cells phagocytose *Cryptococcus neoformans* via mannose receptors and Fcgamma receptor II for presentation to T lymphocytes. *Infect Immun* 2002; 70: 5972–5981.
19. Kelly RM, Chen J, Yauch LE *et al.* Opsonic requirements for dendritic cell-mediated responses to *Cryptococcus neoformans*. *Infect Immun* 2005; 73: 592–598.
20. Mansour MK, Latz E, Levitz SM. *Cryptococcus neoformans* glycoantigens are captured by multiple lectin receptors and presented by dendritic cells. *J Immunol* 2006; 176: 3053–3061.
21. Levitz SM, Nong SH, Seetoo KF *et al.* *Cryptococcus neoformans* resides in an acidic phagolysosome of human macrophages. *Infect Immun* 1999; 67: 885–890.
22. Feldmesser M, Kress Y, Novikoff P *et al.* *Cryptococcus neoformans* is a facultative intracellular pathogen in murine pulmonary infection. *Infect Immun* 2000; 68: 4225–4237.
23. Alvarez M, Casadevall A. Cell-to-cell spread and massive vacuole formation after *Cryptococcus neoformans* infection of murine macrophages. *BMC Immunol* 2007; 8: 16.
24. Del Poeta M. Role of phagocytosis in the virulence of *Cryptococcus neoformans*. *Eukaryot Cell* 2004; 3: 1067–1075.
25. Alvarez M, Casadevall A. Phagosome extrusion and host-cell survival after *Cryptococcus neoformans* phagocytosis by macrophages. *Curr Biol* 2006; 16: 2161–2165.
26. Ma H, Croudace JE, Lammas DA *et al.* Expulsion of live pathogenic yeast by macrophages. *Curr Biol* 2006; 16: 2156–2160.
27. Chaturvedi V, Wong B, Newman SL. Oxidative killing of *Cryptococcus neoformans* by human neutrophils. Evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. *J Immunol* 1996; 156: 3836–3840.
28. Miller MF, Mitchell TG. Killing of *Cryptococcus neoformans* strains by human neutrophils and monocytes. *Infect Immun* 1991; 59: 24–28.
29. Mambula SS, Simons ER, Hastey R *et al.* Human neutrophil-mediated nonoxidative antifungal activity against *Cryptococcus neoformans*. *Infect Immun* 2000; 68: 6257–6264.
30. Lehrer RI, Ganz T. Antimicrobial polypeptides of human neutrophils. *Blood* 1990; 76: 2169–2181.

31. Eisenhauer PB, Lehrer RI. Mouse neutrophils lack defensins. *Infect Immun* 1992; 60: 3446–3447.
32. Houpt DC, Pfrommer GS, Young BJ *et al.* Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. *Infect Immun* 1994; 62: 2857–2864.
33. Fleuridor R, Lyles RH, Pirofski L. Quantitative and qualitative differences in the serum antibody profiles of human immunodeficiency virus-infected persons with and without *Cryptococcus neoformans* meningitis. *J Infect Dis* 1999; 180: 1526–1535.
34. Mukherjee J, Nussbaum G, Scharff MD *et al.* Protective and nonprotective monoclonal antibodies to *Cryptococcus neoformans* originating from one B cell. *J Exp Med* 1995; 181: 405–409.
35. Netski D, Kozel TR. Fc-dependent and Fc-independent opsonization of *Cryptococcus neoformans* by anticapsular monoclonal antibodies: importance of epitope specificity. *Infect Immun* 2002; 70: 2812–2819.
36. Taborda CP, Casadevall A. CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are involved in complement-independent antibody-mediated phagocytosis of *Cryptococcus neoformans*. *Immunity* 2002; 16: 791–802.
37. Levitz SM, Dupont MP, Smail EH. Direct activity of human T lymphocytes and natural killer cells against *Cryptococcus neoformans*. *Infect Immun* 1994; 62: 194–202.
38. Zheng CF, Ma LL, Jones GJ *et al.* Cytotoxic CD4+ T cells use granulysin to kill *Cryptococcus neoformans*, and activation of this pathway is defective in HIV patients. *Blood* 2007; 109: 2049–2057.
39. Ernst WA, Thoma-Uszynski S, Teitelbaum R *et al.* Granulysin, a T cell product, kills bacteria by altering membrane permeability. *J Immunol* 2000; 165: 7102–7108.
40. Voskoboinik I, Smyth MJ, Trapani JA. Perforin-mediated target-cell death and immune homeostasis. *Nat Rev Immunol* 2006; 6: 940–952.
41. Flesch IE, Schwamberger G, Kaufmann SH. Fungicidal activity of IFN-gamma-activated macrophages. Extracellular killing of *Cryptococcus neoformans*. *J Immunol* 1989; 142: 3219–3224.
42. Kawakami K, Kohno S, Kadota J *et al.* T cell-dependent activation of macrophages and enhancement of their phagocytic activity in the lungs of mice inoculated with heat-killed *Cryptococcus neoformans*: involvement of IFN-gamma and its protective effect against cryptococcal infection. *Microbiol Immunol* 1995; 39: 135–143.
43. Milam JE, Herring-Palmer AC, Pandrangi R *et al.* Modulation of the pulmonary type 2 T-cell response to *Cryptococcus neoformans* by intratracheal delivery of a tumor necrosis factor alpha-expressing adenoviral vector. *Infect Immun* 2007; 75: 4951–4958.
44. Kleinschek MA, Muller U, Brodie SJ *et al.* IL-23 enhances the inflammatory cell response in *Cryptococcus neoformans* infection and induces a cytokine pattern distinct from IL-12. *J Immunol* 2006; 176: 1098–1106.
45. Blackstock R, Murphy JW. Role of interleukin-4 in resistance to *Cryptococcus neoformans* infection. *Am J Respir Cell Mol Biol* 2004; 30: 109–117.
46. Muller U, Stenzel W, Kohler G *et al.* IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with *Cryptococcus neoformans*. *J Immunol* 2007; 179: 5367–5377.
47. Kawakami K. Regulation by innate immune T lymphocytes in the host defense against pulmonary infection with *Cryptococcus neoformans*. *Jpn J Infect Dis* 2004; 57: 137–145.
48. Kawakami K, Tohyama M, Xie Q *et al.* IL-12 protects mice against pulmonary and disseminated infection caused by *Cryptococcus neoformans*. *Clin Exp Immunol* 1996; 104: 208–214.
49. Clemons KV, Brummer E, Stevens DA. Cytokine treatment of central nervous system infection: efficacy of interleukin-12 alone and synergy with conventional antifungal therapy in experimental cryptococcosis. *Antimicrob Agents Chemother* 1994; 38: 460–464.
50. Decken K, Kohler G, Palmer-Lehmann K *et al.* Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. *Infect Immun* 1998; 66: 4994–5000.
51. Kawakami K, Qureshi MH, Zhang T *et al.* IL-18 protects mice against pulmonary and disseminated infection with *Cryptococcus neoformans* by inducing IFN-gamma production. *J Immunol* 1997; 159: 5528–5534.
52. Zhang T, Kawakami K, Qureshi MH *et al.* Interleukin-12 (IL-12) and IL-18 synergistically induce the fungicidal activity of murine peritoneal exudate cells against *Cryptococcus neoformans* through production of gamma interferon by natural killer cells. *Infect Immun* 1997; 65: 3594–3599.
53. Qureshi MH, Zhang T, Koguchi Y *et al.* Combined effects of IL-12 and IL-18 on the clinical course and local cytokine production in murine pulmonary infection with *Cryptococcus neoformans*. *Eur J Immunol* 1999; 29: 643–649.
54. Joly V, Saint-Julien L, Carbon C *et al.* In vivo activity of interferon-gamma in combination with amphotericin B in the treatment of experimental cryptococcosis. *J Infect Dis* 1994; 170: 1331–1334.
55. Kawakami K, Tohyama M, Teruya K *et al.* Contribution of interferon-gamma in protecting mice during pulmonary and disseminated infection with *Cryptococcus neoformans*. *FEMS Immunol Med Microbiol* 1996; 13: 123–130.
56. Clemons KV, Lutz JE, Stevens DA. Efficacy of recombinant gamma interferon for treatment of systemic cryptococcosis in SCID mice. *Antimicrob Agents Chemother* 2001; 45: 686–689.
57. Lutz JE, Clemons KV, Stevens DA. Enhancement of antifungal chemotherapy by interferon-gamma in experimental systemic cryptococcosis. *J Antimicrob Chemother* 2000; 46: 437–442.
58. Wormley FL Jr, Perfect JR, Steele C *et al.* Protection against cryptococcosis by using a murine gamma interferon-producing *Cryptococcus neoformans* strain. *Infect Immun* 2007; 75: 1453–1462.
59. Levitz SM, Farrell TP. Growth inhibition of *Cryptococcus neoformans* by cultured human monocytes: role of the capsule, opsonins, the culture surface, and cytokines. *Infect Immun* 1990; 58: 1201–1209.
60. Reardon CC, Kim SJ, Wagner RP *et al.* Interferon-gamma reduces the capacity of human alveolar macrophages to inhibit growth of *Cryptococcus neoformans* in vitro. *Am J Respir Cell Mol Biol* 1996; 15: 711–715.
61. Kawakami K, Qureshi MH, Koguchi Y *et al.* Role of TNF-alpha in the induction of fungicidal activity of mouse peritoneal exudate cells against *Cryptococcus neoformans* by IL-12 and IL-18. *Cell Immunol* 1999; 193: 9–16.
62. Collins HL, Bancroft GJ. Cytokine enhancement of complement-dependent phagocytosis by macrophages: synergy of tumor necrosis factor-alpha and granulocyte–macrophage colony-stimulating factor for phagocytosis of *Cryptococcus neoformans*. *Eur J Immunol* 1992; 22: 1447–1454.
63. Zhou Q, Gault RA, Kozel TR *et al.* Immunomodulation with CD40 stimulation and interleukin-2 protects mice from disseminated cryptococcosis. *Infect Immun* 2006; 74: 2161–2168.
64. Zhou Q, Gault RA, Kozel TR *et al.* Protection from direct cerebral *Cryptococcus* infection by interferon-gamma-dependent activation of microglial cells. *J Immunol* 2007; 178: 5753–5761.
65. Dromer F, Salamero J, Contrepois A *et al.* Production, characterization, and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. *Infect Immun* 1987; 55: 742–748.
66. Shapiro S, Beenhouwer DO, Feldmesser M *et al.* Immunoglobulin G monoclonal antibodies to *Cryptococcus neoformans* protect mice deficient in complement component C3. *Infect Immun* 2002; 70: 2598–2604.
67. Casadevall A, Feldmesser M, Pirofski LA. Induced humoral immunity and vaccination against major human fungal pathogens. *Curr Opin Microbiol* 2002; 5: 386–391.
68. Rodrigues ML, Travassos LR, Miranda KR *et al.* Human antibodies against a purified glucosylceramide from *Cryptococcus neoformans* inhibit cell budding and fungal growth. *Infect Immun* 2000; 68: 7049–7060.

69. Rodrigues ML, Shi L, Barreto-Bergter E *et al.* Monoclonal antibody to fungal glucosylceramide protects mice against lethal *Cryptococcus neoformans* infection. *Clin Vaccine Immunol* 2007; 14: 1372–1376.
70. Rosas AL, Nosanchuk JD, Casadevall A. Passive immunization with melanin-binding monoclonal antibodies prolongs survival of mice with lethal *Cryptococcus neoformans* infection. *Infect Immun* 2001; 69: 3410–3412.
71. Rachini A, Pietrella D, Lupo P *et al.* An anti-beta-glucan monoclonal antibody inhibits growth and capsule formation of *Cryptococcus neoformans* in vitro and exerts therapeutic, anticryptococcal activity in vivo. *Infect Immun* 2007; 75: 5085–5094.
72. Taborda CP, Casadevall A. Immunoglobulin M efficacy against *Cryptococcus neoformans*: mechanism, dose dependence, and prozone-like effects in passive protection experiments. *J Immunol* 2001; 166: 2100–2107.
73. Beenhouwer DO, Shapiro S, Feldmesser M *et al.* Both Th1 and Th2 cytokines affect the ability of monoclonal antibodies to protect mice against *Cryptococcus neoformans*. *Infect Immun* 2001; 69: 6445–6455.
74. Feldmesser M, Mednick A, Casadevall A. Antibody-mediated protection in murine *Cryptococcus neoformans* infection is associated with pleotrophic effects on cytokine and leukocyte responses. *Infect Immun* 2002; 70: 1571–1580.
75. Rivera J, Zaragoza O, Casadevall A. Antibody-mediated protection against *Cryptococcus neoformans* pulmonary infection is dependent on B cells. *Infect Immun* 2005; 73: 1141–1150.
76. Casadevall A, Pirofski LA. A reappraisal of humoral immunity based on mechanisms of antibody-mediated protection against intracellular pathogens. *Adv Immunol* 2006; 91: 1–44.
77. Taborda CP, Rivera J, Zaragoza O *et al.* More is not necessarily better: prozone-like effects in passive immunization with IgG. *J Immunol* 2003; 170: 3621–3630.
78. Beenhouwer DO, Yoo EM, Lai CW *et al.* Human immunoglobulin G2 (IgG2) and IgG4, but not IgG1 or IgG3, protect mice against *Cryptococcus neoformans* infection. *Infect Immun* 2007; 75: 1424–1435.
79. Yuan R, Casadevall A, Spira G *et al.* Isotype switching from IgG3 to IgG1 converts a nonprotective murine antibody to *Cryptococcus neoformans* into a protective antibody. *J Immunol* 1995; 154: 1810–1816.
80. Yuan RR, Spira G, Oh J *et al.* Isotype switching increases efficacy of antibody protection against *Cryptococcus neoformans* infection in mice. *Infect Immun* 1998; 66: 1057–1062.
81. Yuan RR, Casadevall A, Oh J *et al.* T cells cooperate with passive antibody to modify *Cryptococcus neoformans* infection in mice. *Proc Natl Acad Sci USA* 1997; 94: 2483–2488.
82. Rivera J, Mukherjee J, Weiss LM *et al.* Antibody efficacy in murine pulmonary *Cryptococcus neoformans* infection: a role for nitric oxide. *J Immunol* 2002; 168: 3419–3427.
83. Fleuridor R, Zhong Z, Pirofski L. A human IgM monoclonal antibody prolongs survival of mice with lethal cryptococcosis. *J Infect Dis* 1998; 178: 1213–1216.
84. Dromer F, Charreire J, Contrepois A *et al.* Protection of mice against experimental cryptococcosis by anti- *Cryptococcus neoformans* monoclonal antibody. *Infect Immun* 1987; 55: 749–752.
85. Mukherjee J, Scharff MD, Casadevall A. Protective murine monoclonal antibodies to *Cryptococcus neoformans*. *Infect Immun* 1992; 60: 4534–4541.
86. Sanford JE, Lupan DM, Schlageter AM *et al.* Passive immunization against *Cryptococcus neoformans* with an isotype-switch family of monoclonal antibodies reactive with cryptococcal polysaccharide. *Infect Immun* 1990; 58: 1919–1923.
87. Casadevall A, Cleare W, Feldmesser M *et al.* Characterization of a murine monoclonal antibody to *Cryptococcus neoformans* polysaccharide that is a candidate for human therapeutic studies. *Antimicrob Agents Chemother* 1998; 42: 1437–1446.
88. Dadachova E, Nakouzi A, Bryan RA *et al.* Ionizing radiation delivered by specific antibody is therapeutic against a fungal infection. *Proc Natl Acad Sci USA* 2003; 100: 10942–10947.
89. Dadachova E, Bryan RA, Apostolidis C *et al.* Interaction of radiolabeled antibodies with fungal cells and components of the immune system in vitro and during radioimmunotherapy for experimental fungal infection. *J Infect Dis* 2006; 193: 1427–1436.
90. Dadachova E, Bryan RA, Frenkel A *et al.* Evaluation of acute hematologic and long-term pulmonary toxicities of radioimmunotherapy of *Cryptococcus neoformans* infection in murine models. *Antimicrob Agents Chemother* 2004; 48: 1004–1006.
91. Bryan RA, Jiang Z, Huang X *et al.* Radioimmunotherapy is effective against high-inoculum *Cryptococcus neoformans* infection in mice and does not select for radiation-resistant cryptococcal cells. *Antimicrob Agents Chemother* 2009; 53: 1679–1682.
92. Bryan RA, Jiang Z, Howell RC *et al.* Radioimmunotherapy is more effective than antifungal treatment in experimental cryptococcal infection. *J Infect Dis* 2010; 202: 633–637.
93. Siddiqui AA, Brouwer AE, Wuthiekanun V *et al.* IFN-gamma at the site of infection determines rate of clearance of infection in cryptococcal meningitis. *J Immunol* 2005; 174: 1746–1750.
94. Brouwer AE, Siddiqui AA, Kester MI *et al.* Immune dysfunction in HIV-seronegative, *Cryptococcus gattii* meningitis. *J Infect* 2007; 54: e165–e168.
95. Lortholary O, Dromer F, Mathoulin-Pelissier S *et al.* Immune mediators in cerebrospinal fluid during cryptococcosis are influenced by meningeal involvement and human immunodeficiency virus serostatus. *J Infect Dis* 2001; 183: 294–302.
96. Pappas PG, Bustamante B, Ticona E *et al.* Recombinant interferon-gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. *J Infect Dis* 2004; 189: 2185–2191.
97. Netea MG, Brouwer AE, Hoogendoorn EH *et al.* Two patients with cryptococcal meningitis and idiopathic CD4 lymphopenia: defective cytokine production and reversal by recombinant interferon-gamma therapy. *Clin Infect Dis* 2004; 39: e83–e87.
98. Larsen RA, Pappas PG, Perfect J *et al.* Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. *Antimicrob Agents Chemother* 2005; 49: 952–958.
99. Perfect JR, Dismukes WE, Dromer F *et al.* Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010; 50: 291–322.
100. Pirofski LA, Casadevall A. Immunomodulators as an antimicrobial tool. *Curr Opin Microbiol* 2006; 9: 489–495.
101. Huang H, Ostroff GR, Lee CK, Specht CA, Levitz SM. Robust stimulation of humoral and cellular immune responses following vaccination with antigen-loaded beta-glucan particles. *MBio* 2010; 1: pii: e00164–10.